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Short Communication

Taxonomic studies and DNA barcoding of sapota bud borer, *Eustalodes achrasella* (Bradley, 1981) (Lepidoptera: Gelechiidae) from India

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ABSTRACT

The sapota bud borer, *Eustalodes achrasella* (Bradley, 1981) (Lepidoptera: Gelechiidae), causes significant damage in the commercial sapota orchards leading to economic yield losses. The young larvae damage the foliage and buds by webbing and scraping the tissue, whereas, later stages bore into fruits resulting in severe drying, defoliation and reduced crop yields. The present study investigated the field infestation and damage symptoms caused by *E. achrasella* in Punjab, India. It also includes illustrations of various life stages of the pest, a detailed taxonomic redescription, and the development of a novel mitochondrial cytochrome oxidase I DNA barcode for precise identification of this species.

Keywords: Buds, *Eustalodes*, fruits, larval feeding, mtCO1, sapotaceae, webbing, yield loss

INTRODUCTION

Manilkara zapota (L.) P. Royen, (Sapotaceae), commonly known as sapodilla or sapota, holds a significant place as a tropical evergreen fruit crop. Originating from southern Mexico and Central America, it has spread to various countries like Pakistan, India, Sri Lanka, Thailand, Malaysia, Cambodia, Indonesia, Vietnam, Bangladesh and the Caribbean. India has emerged as the foremost producer, highlighting its widespread cultivation and economic importance (Venkateswara et al., 2014; Martinez et al., 2017). Though many insects are reported to attack sapota (Clarke, 1954; Butani, 1975; Bradley, 1981; Medina-Goud et al., 1987; Witethom & Silawatchananai, 1990; Iruegas et al., 2002; Martinez et al., 2017; Bisane, 2018), the lepidopteran pests pose a significant threat to its cultivation. Around thirty-four species of Lepidoptera have been identified as pests with notable species, *Banisia myrsusalis* Walker (Thyrididae), *Eustalodes achrasella* Bradley (Gelechiidae) and *Nephopteryx eugraphella* (Ragonot) (Pyralidae), responsible for the yield loss (Martinez et al., 2017; Bisane et al., 2019; Patil & Kumari, 2023).

Among them, *E. achrasella* (Bradley, 1981), has been reported from several states of India, including Chhattisgarh, Gujarat, Karnataka, Punjab and Tamil Nadu (Sandhu & Sran, 1980; Bradley, 1981; Shah et al., 1986; Parvathi & Belavadi, 1994; Jothi & Tandon, 1994; Sathish et al., 2014; Ghirtlahre et al., 2016; Bisane et al., 2019). This species is a key pest impacting sapota yield potential, significantly reducing both productivity and quality (Bisane, 2018). *N. eugraphella* and *E. achrasella* affect sapota yields in Gujarat, damaging 20 to 30 per cent of flowers and buds (Jhala et al., 1988; Bisane et al., 2019). Similarly, Jayanthi & Verghese (2003) reported that *E. achrasella* can damage between 36.9 to 46.6 per cent of buds before reaching pupation.

For a notable pest like *E. achrasella*, there is a lack of detailed morphological descriptions including genitalia descriptions with photographic illustrations. Also, the molecular marker, mitochondrial cytochrome c oxidase subunit I (mtCOI), is regarded as a useful tool for identifying and characterizing important insect species (Shashank et al., 2022). Currently, there are no DNA barcodes available for this species to confirm the identity of *E. achrasella*. Hence, this study, provided damage symptoms, detailed morphological redescriptions with male genitalia illustrations and voucher-based DNA barcodes for the *E. achrasella* using the mtCOI gene.

E. achrasella was observed from the first week of April to the last week of August in both 2022 and 2023 at the College Orchard, Punjab Agricultural University (PAU), Ludhiana, India. The different life stages of

E. achrasella were collected and reared under laboratory conditions at 25±2°C and 70±5% relative humidity at the Department of Fruit Science, PAU, Ludhiana, India. The specimens were carefully pinned and deposited at Indian Agricultural Research Institute- National Pusa Collection (INPC), Division of Entomology, Indian Agricultural Research Institute (IARI), New Delhi, India.

The genitalia slides were prepared following the procedure given by Huemer & Karsholt (1999). For wing venation studies, the right wings were carefully detached and immersed in eosin stain overnight. The scales and excess stain were then cleaned with a camel hair brush (size 000) in absolute ethanol. The terminologies of genitalia and wing venation were in accordance with Kristensen (2003). Photography of adult moths, wings and male genitalia was conducted at various magnifications using a Leica EC4 digital camera mounted on a Leica M205FA stereozoom automontage microscope and subsequently deposited at the INPC, Division of Entomology, IARI, New Delhi for future reference.

DNA was extracted from the specimen using Qiagen DNeasy® Blood and tissue kit following manufacturer's protocol (Qiagen, Valencia) at INPC, IARI, New Delhi and subjected to PCR amplification using the primers, LCO 1490 and HCO 2198, following Shashank et al. (2015). The amplified products were quantified, sequenced and submitted to National Center for Biotechnology Information (NCBI).

The infestation was observed on flower buds and young fruits from the first week of April to the last week of August during 2022 and 2023. The damage was observed to be higher during summer, with 13.34% in var. Kalipatti, compared to other varieties studied (Solanki & Bisane, 2021). On the other hand, minimum temperature accounted for about 53% variability in bud borer incidence (Jayanthi et al., 2006).

Adult females laid eggs during the first and second weeks of April and first instar larvae were subsequently observed in the folded newly emerged leaves. The larva is light-coloured with brownish-orange head and dark brownish thorax marked by a distinctive white transverse band. The larvae exhibit different types of feeding behaviour, such as webbing and defoliation of leaves, feeding on floral buds and boring into fruits. Initially, the larvae fed on the leaf lamina by folding the leaves, scraping and making irregular holes in the young foliage (Fig. 1A, B and C). They caused webbing of the flower buds as they grow and continued feeding internally, causing bud drop and failure in the fruit set (Fig. 1D and E). A single larva can damage up to 37 buds by feeding on the ovary and petals before reaching pupation, adversely affecting sapota production (Jayanthi et al., 2006).

In the later stages, they bore into the fruit leading to a white gummy exudation from the fruit (Fig.1F). The inactive prepupal (Fig. 1G) and pupal stages were found within webbed leaves or buds, occasionally on sapota fruit (Fig.1H, I and J). The adult moths are small and dark greyish, characterized by upcurved labial palpi and greyish fringes on hind wings (Fig.1 K and L).

Taxonomic Redescription

Eustalodes achrasella (Bradley, 1981)

Anarsia achrasella Bradley, 1981: 617; TL: Mirpur, Sakro, Pakistan (Holotype B&) (NHMUK).

Head: Smooth scaled, compound eyes greyish black. Vertex and frons covered with pale grey scales; labial palpus three segmented; second segment with long coarse scales projecting forward; third segment vestigial in males (Fig. 2C) whereas long, curved and covered in feathery black scales, tapering to a pointed grey tip in female (Fig. 2D). Antennae filiform, nearly as long as forewing, light greyscales.

Thorax: Light brown with grey white scales; wingspan 12-14 mm (N=16); Forewing whitish grey with band of dark grey irroration along costal margin and irregularly scattered with black and brown scales, black hair-pencils or androconial patch on underside of wings arising from base and reaching middle in males (Fig. 3A); apex acute, termen relatively concave margin, tornus indistinct with long cilia, anal margin convex; hind-wing greyish with light grey cilia; costa strongly convex at basal 2/3rd; Forewing R_4 and R_5 stalked with M_1 approximate. Hindwing Sc and R_1 veins run in close approximate, Rs and M_1 veins rise from same base (Fig. 3B).

Abdomen: Light brown with yellowish white scales with anal tuft of pale ochreous hairs. Male genitalia (Fig. 3C) Uncus sub rectangular with two symmetrical spine-like projections at median distal margin and long

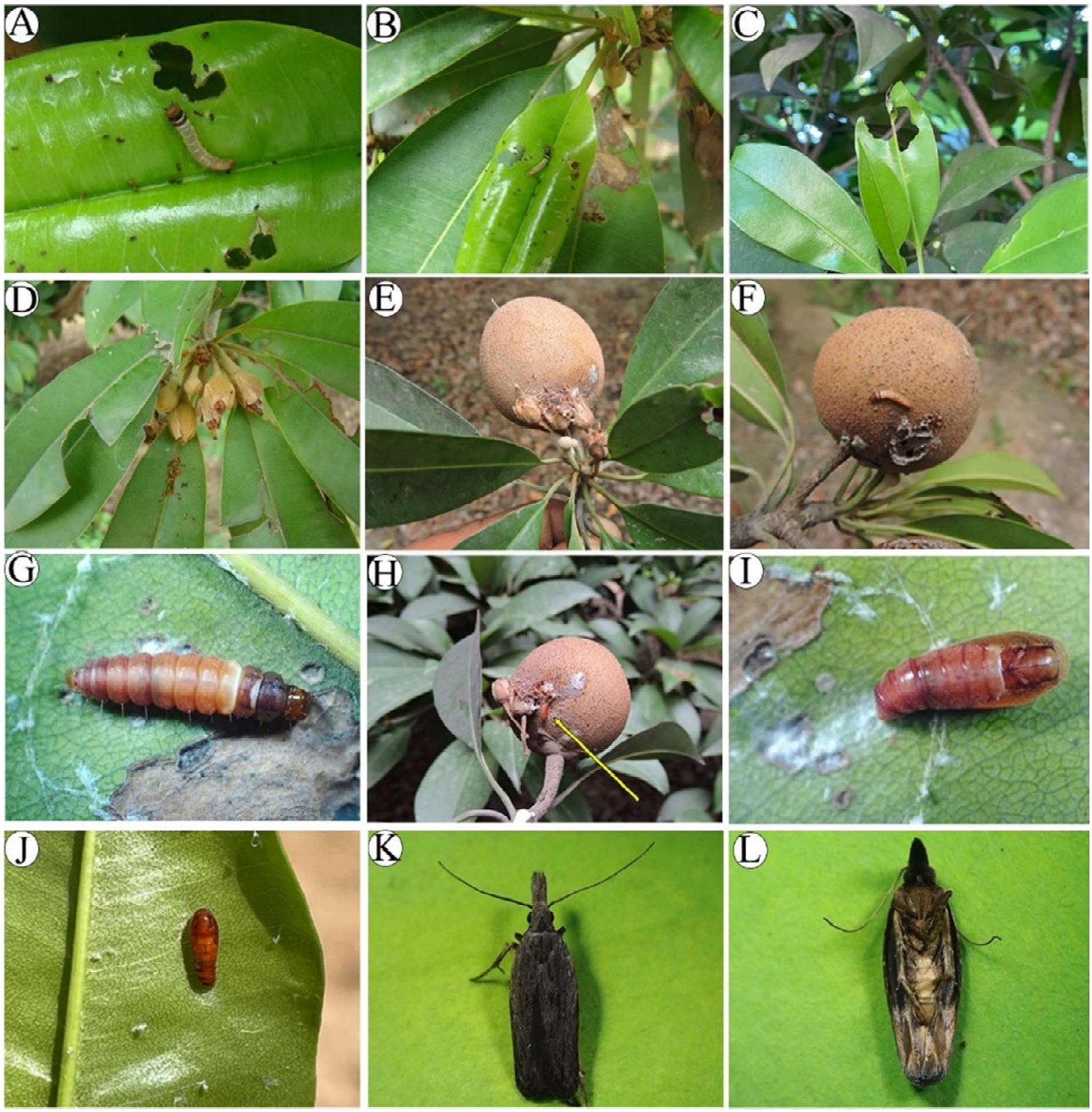

Fig. 1 : A and B. Larva recorded on leaves of sapota; C. Feeding symptoms on leaves; D. Damage of the unopened floral buds; E. Webbing of the young floral buds; F. Borehole on the fruit leading to gummy white exudation; G. Prepupal stage of *E. achrasella*; H. Pupa of *E. achrasella* on fruit; I. and J. Pupa of *E. achrasella* on leaves of sapota; K. Dorsal view of *E. achrasella* male adult; L. Ventral view of *E. achrasella* male adult

fringes along margins except proximal margin; gnathal hook sickle shaped, heavily sclerotized, arising at base of uncus and about 2/3rd length of uncus; tegumen broader at base, narrower towards uncus and folded along lateral and convex margin; valva simple, narrow with parallel margin and fringed relatively foot shaped distal dilation; sacculus swollen with a curved terminal hook at base of left valva; phallus short, curved, wider at base like a bulb, tapers and curves towards tip (Fig. 3D).

Female genitalia (Fig. 3E) Posterior apophyses long and anterior apophysis not developed; Ductus bursae slender and long. Corpus bursae long, slightly globose; signum cross-shaped with tapering ends (Fig. 3F).

Material examined: India, Punjab, Ludhiana (30°54'14" N, 75°48'23" E, 247 mts MSL),

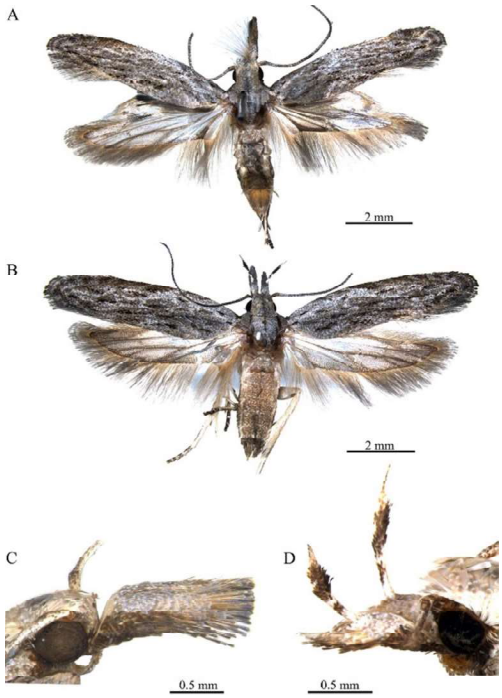

Fig. 2 : A. Male habitus of *E. achrasella*;
B. Female habitus of *E. achrasella*;
C. Labial palpi of male;
D. Labial palpi of female

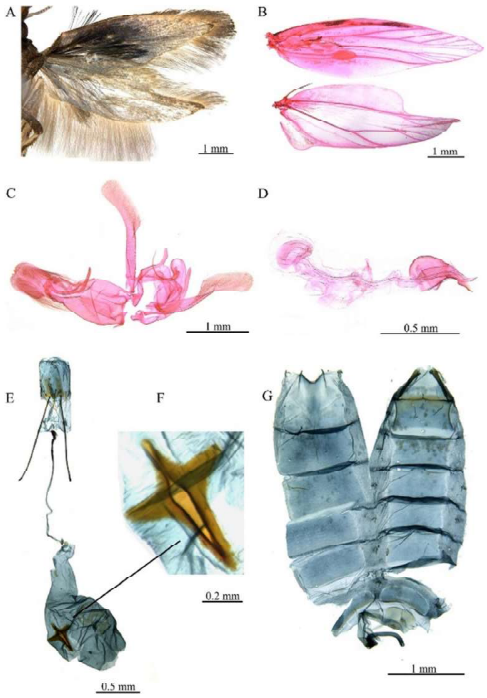

Fig. 3 : A. Hair pencils on the ventral surface of forewing; B. Wing venation (forewing+hind wing);
C. Male genitalia of *E. achrasella*; D. Phallus;
E. Female genitalia of *E. achrasella;* F. Signum;
G. Abdomen of female

6♂, 23.vi.2023, *Manilkara zapota*, Coll. Sandeep Singh (genitalia --slide no. LS00055101–LS00055104 (♂); INPC); New Delhi, IARI (28°04'48" N, 77°07'12"E, 229 mts MSL), ♂ and 6♀, 24.ix.2024, Mercury vapour lamp, Coll. Nandhini and Sudipa (genitalia slide no. LS00055105– LS00055106 (♀); INPC)

Distribution: Gujarat (Navsari), Punjab (Ludhiana) (Bradley, 1981); Chhattisgarh (Ghirtlahre et al., 2016); Karnataka (Parvathi & Belavadi, 1994); New Delhi (present study). Elsewhere: Pakistan (Bradley, 1981).

Remarks: Bradley (1981) initially described this species under genus *Anarsia* Zeller, 1839. However, Ponomarenko (1997) reassigned the species to the genus *Eustalodes* Sattler, 1973.

The mtCOI gene was sequenced, submitted to the NCBI GenBank and assigned the accession numbers PP907948 and PP907949. The corresponding voucher specimens are preserved in the INPC (http://grbio.org/cool/nwky-2b63), Division of Entomology, ICAR-IARI, New Delhi. BLAST analysis of sequences indicated that the taxa matched with the genus *Anarsia*. Currently, there is no DNA barcode available for *E. achrasella* in public databases such as BOLD and GenBank, establishing the sequences from this study as novel contributions to the genetic barcode repository. Overall, the observed infestation patterns highlight the need for vigilant monitoring and management practices to mitigate the impact of bud borer larvae on sapota crops, particularly during their peak activity period.

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